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VOL. 25, SEC. E. OCTOBER, 1947 NUMBER 5

OBSERVATIONS ON SHOCK PRODUCED IN THE RAT BY LIMB ISCHAEMIA[1]

BY R. L. NOBLE[2] AND C. GWENDOLINE TOBY[3]

Abstract

Experimental shock has been produced in the rat by clamping the hind legs. The mortality was related to the duration of limb ischaemia, as were the increased haemoglobin values. Adrenalectomized animals showed much greater susceptibility to trauma than did intact animals; DCA afforded no protection against trauma whereas adrenal cortical extract considerably improved the lowered tolerance. Conclusive evidence of development of resistance following repeated clamping was not obtained. Rats that had previously received a single clamping showed little evidence of resistance to drum trauma, whereas animals resistant to drumming showed increased tolerance when subjected to clamping.

A quantitative method for the administration of experimental trauma in the rat has been previously described by Noble and Collip (4). Using this rotating drum method, studies on the influence of the adrenal glands on mortality from shock (5) and on factors affecting the development of resistance to trauma induced by repeated traumatization have been reported (3, 7). Since, by this method, rats could be made to withstand trauma in amounts ordinarily fatal, it seemed of interest to determine whether such resistance would afford protection against a different type of trauma. For such experiments, the method described by Haist and Hamilton was employed (1). This consists of applying clamps to the hind legs of the rat to cut off the circulation in these areas. After a sufficiently long period of ischaemia, mortality follows release of the clamps. The susceptibility of normal animals, of untreated and treated adrenalectomized animals, and of animals previously made resistant to drum trauma was investigated. The percentage of mortality, the time of death following various lengths of time of clamping, and the changes in haemoglobin levels as evidence of haemoconcentration are reported. Experiments were devised to develop increased tolerance to clamping and to demonstrate crossed resistance.

Methods

Adult hooded rats of a strain maintained in this laboratory, and weighing between 160 and 200 gm. were used in these experiments. The stock diet consisted of Purina fox chow supplemented with brown bread twice a week.

[1] *Manuscript received June 24, 1947.*

Contribution from the Research Institute of Endocrinology, McGill University, Montreal, Que., with financial assistance from the National Research Council of Canada.

[2] *Associate Professor, Research Institute of Endocrinology.*

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The adrenalectomized animals were given 0.9% saline to drink or were maintained by injections of desoxycorticosterone acetate (DCA) or of adrenal cortical extract (ACE, Connaught Laboratories, 30 dog units per cc.). When stock rats of this colony and of the age used in these experiments are adrenalectomized, approximately 40% survive when given water to drink. Inspection does not reveal any gross adrenal tissue. A series of experiments was also conducted using animals maintained on a protein-free diet, consisting of cornstarch and glucose with 6% lard and 4% salt mixture added. The clamping method used was essentially that described by Haist and Hamilton (1). The rats were lightly anaesthetized with ether, suspended in slings, and the metal clamps tightened by hand, always by the same individual. Following the release of the clamps, the animals were allowed access to food and water. They were kept at room temperature. Trauma by the drum method was administered as described previously (4) and rats were made resistant by repeatedly exposing them to increasing amounts of this type of trauma. Haemoglobin levels were measured in the Evelyn photoelectric colorimeter, using 0.02 cc. of blood obtained from the tail.

Results

Effect of Clamping Without Treatment

The percentage of mortality following various lengths of time of clamping of from 1 to 10 hr. is shown in Table I. It might be noted that little difference

TABLE I

PERCENTAGE OF MORTALITY FOLLOWING LIMB ISCHAEMIA

Types of rats	Hours of clamping							
	1	1½	3	4	5	6	6½ to 7	8 to 10
Normal—both legs clamped	0 (8)*		0 (50)	0 (4)	50 (18)		70 (30)	76 (26)
Normal—one leg clamped						0 (12)		
Adrenalectomized	0 (4)	23 (13)	43 (21)					
Adrenalectomized + DCA			37 (18)					
Adrenalectomized + ACE two days**			6 (18)					
Adrenalectomized + ACE six days***			18 (11)					
Left adrenal only removed			0 (9)					
Drum resistant							50 (18)	

* *Figures in parentheses give number of rats.*

** *0.5 cc. × 3 on day preceding and 0.5 cc. × 6 during experiment.*

*** *0.5 cc. t.i.d.*

in susceptibility to clamping was found between males and females, but animals not differing greatly in weight were used in the majority of the experiments, so that the mass of the ischaemic muscle was presumably fairly constant. The resistant animals, because of the time required to achieve this

state, were of necessity somewhat heavier. The total figures in the larger series are made up of groups of 6 to 10 animals run at different times over a considerable period. In normal rats, no deaths occurred following four hours or less of clamping. After five hours of clamping, the mortality curve rose sharply to 50%, and after 8 to 10 hr. of ischaemia, 76% of the animals died. No deaths occurred in rats that had only one leg clamped for six hours. Forty per cent of the deaths occurred within four hours of releasing the clamps, and the rest in from 7 to 48 hr. Within a few minutes of removing the clamps the circulation appeared to be restored, the limbs became pink and swelling soon occurred. In some instances, following very long ischaemia, the animals survived and there appeared to be permanent occlusion of the vessels—the legs remained blue, no swelling occurred, and the tissues later became necrotic. Such cases were not included in the results.

Effect of Adrenalectomy and Treatment with DCA and ACE

The greatly increased susceptibility of adrenalectomized rats to clamping is also shown in Table I. The time after operation made little difference. Even when clamps were applied immediately, mortality was high. When those surviving the trauma were given water instead of saline to drink, fewer than 50% survived. Adrenalectomized rats are approximately twice as susceptible to clamping as are intact animals, since after one and a half hours of clamping, 23% of the adrenalectomized rats died, and after three hours, 43%. The survival time of adrenalectomized rats was noticeably shorter than that of intact animals. One mgm. of DCA given daily subcutaneously to adrenalectomized rats gave little or no increased protection against shock. Animals treated with ACE were considerably more resistant, although their tolerance did not approach that of the intact animals. In one series 0.5 cc. of ACE was injected subcutaneously three times on the day before the experiment and six times the following day, beginning immediately after the clamps were removed. In this group, the mortality was 6%. When adrenalectomized rats were maintained from operation by the injection of 0.5 cc. of ACE three times daily the mortality was 18%.

Attempts to Induce Resistance to Clamping

Attempts were made to obtain increased tolerance to clamping, by using repeated clampings of short but increasing duration, based on the principle used for obtaining drum resistant animals. In preliminary experiments, of 10 rats clamped initially for one or three hours and then after an interval of three days reclamped for eight hours, nine succumbed within three hours and the 10th animal died within 48 hr. Of four rats that did not receive a second clamping until 18 days later, two survived and two died at 18 and 48 hr. after re-establishment of the circulation. In another series, 24 rats were clamped repeatedly at intervals of from three to seven days, beginning with 15 min. clamping and increasing the time to 30 min., one, three, five, seven, eight, and nine hours. Five out of 10 rats survived seven hours of clamping. Little increase in tolerance was demonstrated, except in one group of five rats that

all survived nine hours of clamping. In this group, the intervals between traumata were longer than in the other groups. In all these experiments the survival time was in most instances shorter than that of control animals receiving only a single clamping.

Haemoglobin Investigations

Following clampings of different durations, haemoglobin estimations were done on some groups of animals, at intervals of 1, 3, 6, and 19 to 24 hr. after removal of the clamps. Haemoglobin determinations done on each animal just before clamping were taken as 100%. The results are shown in Table II.

TABLE II

AVERAGE HAEMOGLOBIN CHANGES FOLLOWING CLAMPING

No. rats	Length of time of clamping, hr.	Haemoglobin, %				
		Control	1 hr.	3 hr.	6 hr.	19 to 24 hr.
6	1	100	110	107	103	92
6	3	100	124	132	130	105
4	5	100	118	129	129	—
4	7	100	117	128	—	122
8	8	100	129	138	143	142

One hour of clamping produced little haemoconcentration. After three to five hours of ischaemia, the haemoglobin increased about 30% reaching its peak at about three hours and remaining at this level for at least three hours. It returned to normal within 24 hr. Following eight hours of clamping, the increase was greater and the peak was reached more slowly. In these animals, haemoconcentration persisted for 24 hr. or more, in some cases only returning to normal on the second day. A group of repeatedly clamped rats, when subjected to eight hours' clamping, showed an increase of 56% over their control levels. The mortality in this group was the same as that of previously non-clamped rats, but the survival time was shorter. Groups of animals having the highest haemoglobin values appeared to show the greatest swelling of the hind legs.

Attempts at Crossed Resistance

Rats that were made resistant to drum trauma, by the method previously described (3), showed an increased tolerance to clamping. Mixed groups of normal and resistant animals were clamped for six and a half or seven hours. Of the 26 controls, 23% survived, whereas of the 18 resistant animals, 50% survived. The time of death of those resistant rats that succumbed was more delayed than that of the controls. The survivors did not lose their resistance to drum trauma.

In the first series of experiments in which rats, previously clamped, were traumatized in the drum, no increase in resistance was found. Eight out of

10 rats, clamped one to three hours, died one to six days later when given sufficient trauma in the drum to cause 80% to 100% mortality in normal animals. Eight rats that survived repeated clampings were given drum trauma 6 to 13 days later and all but one died.

Effect of Diet

In other experiments, a larger number of rats were maintained on a protein-free diet and then clamped. Following this, they were kept on the same diet and later traumatized in the drum. Of 53 rats drummed three days after clamping, the mortality was somewhat lower than that anticipated from control experiments. When the interval between the different forms of trauma was increased to five to seven days, the mortality was not appreciably reduced. Further studies on the effect of diet on susceptibility to shock following trauma are to be reported in a separate paper.

Discussion

It is apparent from these experiments that mortality following clamping is dependent on the length of time the limbs have been kept ischaemic and on the muscle mass involved. When mortality is high, the average survival time is shorter. Haist and Hamilton (1) using Wistar rats kept at 27° C. found that 12 to 15 hr. of clamping gave 100% mortality with the average time of death occurring at three hours after removal of the clamps, and maximum swelling at one to two hours.

Adrenalectomized rats, which have already been reported to be more susceptible to shock after drum trauma (5), were also more susceptible following limb ischaemia. This appeared to be true even when clamps were applied immediately after adrenalectomy. Although there was some added trauma from operation, it is believed that depletion of circulating adrenal cortical hormone led to the rapid reduction in tolerance. Treatment with DCA conferred no protection, whereas ACE considerably increased the tolerance to shock. The ACE given previous to the clamping appeared to serve this purpose as well as the larger amounts administered later.

In the experiments designed to induce resistance by repeated clamping, little positive evidence was obtained except in two experiments in which the intervals between clamping were considerably longer. In one instance, a second clamping after 18 days appeared to reduce mortality from 76% to 50%. Locke (2) reported increased local resistance following a single clamping two weeks previously. He concluded that the protection was due to muscle atrophy since, in another series in which first one leg was clamped and two weeks later the second leg was clamped for 12 hr., there was no evidence of increased resistance. In a more recent report on acquired resistance to trauma in the mouse, Rosenthal, Tabor, and Lillie (6) confirmed the finding of Locke (2) that the resistance developed following tourniquet application is local, and that when a second tourniquet application is applied to a new site,

there is no difference in mortality between previously clamped and non-clamped groups.

Haemoconcentration appears to be a major finding in this type of shock, the severity and duration depending on the length of time the limbs have remained ischaemic. Haemoconcentration was found to be greater in previously clamped animals. This is in keeping with Locke's finding (2) that water loss into the damaged area was greater under such conditions. Both Locke (2) and Rosenthal, Tabor, and Lillie (6) conclude that fluid loss cannot in itself be the cause of death. This agrees with experiments already reported (4) in which rats dying following drum trauma show far less haemoconcentration than many of those surviving long intervals of clamping.

Experiments to demonstrate crossed resistance indicated that previously clamped animals showed some evidence of increased tolerance to drum trauma. Since the animals used were not resistant to clamping, a high degree of tolerance to the drum could not be expected. On the other hand, rats that had become resistant to amounts of drum trauma ordinarily fatal, showed greater evidence of crossed resistance when subjected to clamping.

Acknowledgments

The authors wish to thank Prof. J. B. Collip for his helpful interest in this problem. Mr. E. Pedersen and Mr. R. Rasmussen rendered valuable technical assistance.

References

1. HAIST, R. E. and HAMILTON, J. I. J. Physiol. 102 : 471-483. 1944.
2. LOCKE, W. J. Report of Committee on Naval Medical Research. Appendix B, C4041. 1943.
3. NOBLE, R. L. Am. J. Physiol. 138 : 346-351. 1943.
4. NOBLE, R. L. and COLLIP, J. B. Quart. J. Exptl. Physiol. 31 : 187-199. 1942.
5. NOBLE, R. L. and COLLIP, J. B. Quart. J. Exptl. Physiol. 31 : 201-210. 1942.
6. ROSENTHAL, S. M., TABOR, H., and LILLIE, R. D. Am. J. Physiol. 143 : 402-406. 1945.
7. TOBY, C. G. and NOBLE, R. L. Can. J. Research, E, 22 : 79-88. 1944.

THE PULMONARY FUNGUS, *HAPLOSPORANGIUM PARVUM*, AND ITS RELATIONSHIP WITH SOME HUMAN PATHOGENS[1]

By Eleanor Silver Dowding[2]

Abstract

In a survey of the wild rodents of Alberta it was found that 14 animals contained within their lungs fungous cells so large as to be visible to the naked eye. From the lungs of eight of these infected animals, *Haplosporangium parvum* was obtained in culture. The position of the fungus among the Phycomycetes is confirmed by a study of the reproductive structures, which may be interpreted as sporangia containing one or, rarely, several spores. *H. parvum* grows and sporulates upon soil. The sporangia are adhesive and transferred by contact. At 37° C. they enlarge as much as 10 times their original diameter to form thick-walled chlamydospores that will germinate at room temperature. The parasite within the lung has a diameter as much as 50 times that of the sporangium from which it must have originated. Infected lungs usually show little reaction. Only one lung was frankly granulomatous. Some human systemic Phycomycetes, like *H. parvum*, are filamentous in their saprophytic phase and unicellular in their parasitic phase. Three of them, *Blastomyces dermatiditis*, *B. brasiliensis*, and *Histoplasma capsulatum*, are closely related to *H. parvum*. In their saprophytic phase they all reproduce by adhesive conidia that may be interpreted as sporangia with single endospores, while in their parasitic phase no sporangia have been observed. *Coccidioides immitis* is less closely related to *H. parvum*. When growing saprophytically it reproduces by air-borne oidia; when growing parasitically, by endospores.

A Search for *Coccidioides* and *Haplosporangium* in Alberta Rodents

Emmons (7) has shown that the small desert rodents of Arizona are a natural reservoir for *Coccidioides immitis* Rixford and Gilchrist, the systemic fungus responsible for valley fever in California. From the lungs of *Perognathus*, *Dipodomys*, *Citellus*, and other rodents near San Carlos, Ariz., Emmons and Ashburn (9) isolated and described for the first time a closely related Phycomycete, *Haplosporangium parvum*.

The demonstration that rodents are capable of acting as reservoirs for systemic fungi suggested that the presence or absence in any locality of certain human pathogens might be conveniently determined by an examination of its wild rodents.

During the summer of 1946 the Entomological Survey of the Department of Public Health collected lungs of Alberta rodents. An area of about 1000 sq. miles was covered, from Lethbridge in the south to Peace River in the north. The localities from which collections were made are shown in Fig. 1. Two hundred and seventy-five animals (eight species) were shot or trapped. The eight species are listed in Table I.

[1] *Manuscript received April 15, 1947.*
Contribution from the Provincial Laboratory, University of Alberta, Edmonton, Alta.
[2] *Medical Mycologist.*

In the field an autopsy was made of each animal. A portion of the lungs was preserved in formalin and another portion was transferred, under as sterile conditions as possible, to Sabouraud's medium. The results of the laboratory examination of the tissues and cultures so obtained have been published in brief (6). They are now described in more detail.

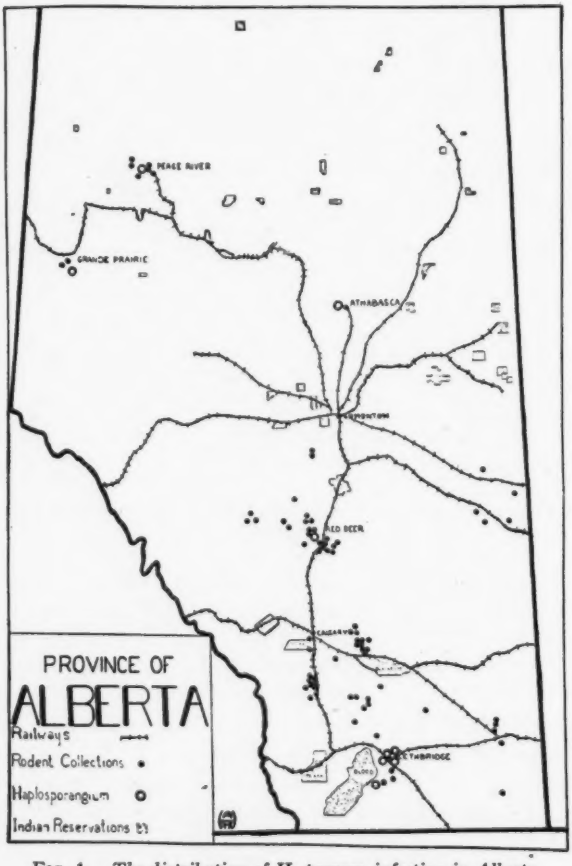

FIG. 1. *The distribution of H. parvum infection in Alberta.*

Of the 275 animals examined, the lungs of two of them (one white-footed deer mouse, *Peromyscus maniculatus borealis*, and one harvest mouse, *Reithrodontomys humulis* (Table I)) yielded fungi resembling *Coccidioides immitis* in general appearance and in the spores (oidia). However, these did not prove to be pathogenic. Furthermore, nine experimental white mice were inoculated intraperitoneally with the two 'coccidioidal' fungi, and three weeks later the animals were still healthy. Upon autopsy their lungs contained no

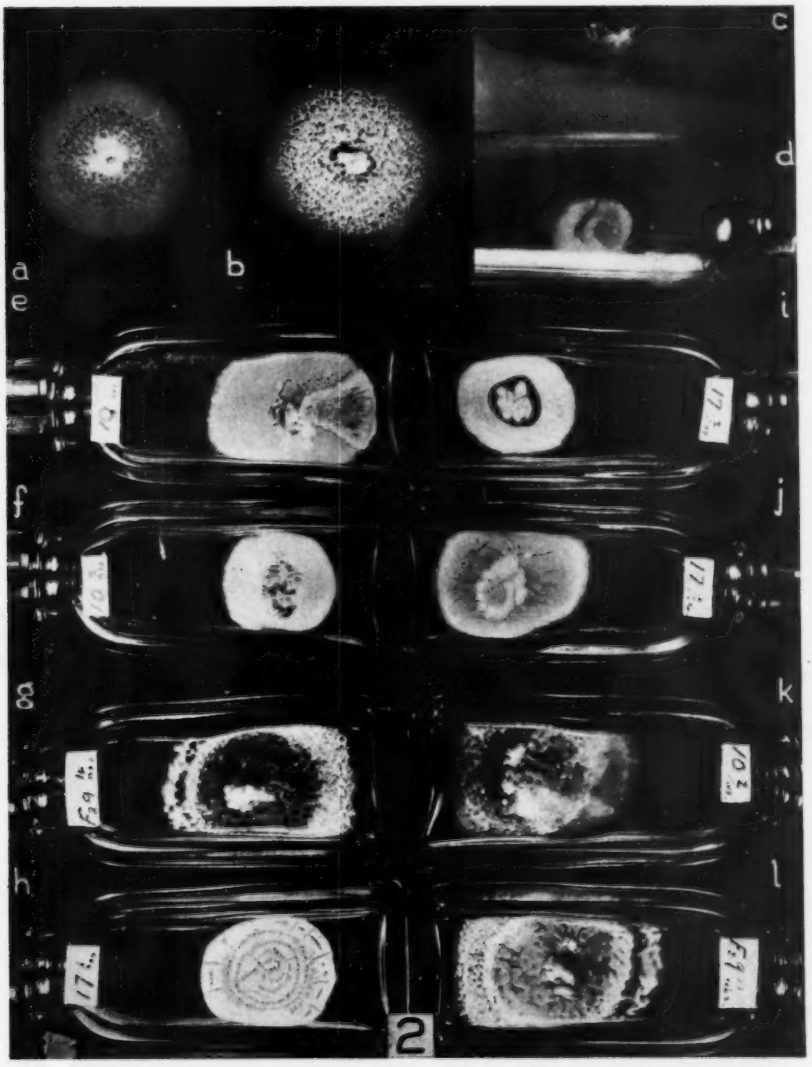

FIG. 2. *H. parvum cultures: a to d, under two weeks old; e to l, more than two weeks old. Natural size.*

FIG. 3. *Sporangia and chlamydospores of H. parvum: a and b, sporangia; c, chlamydospores; d, germinating sporangia; e, germinating chlamydospore (a, b, and c, Diehl mounts; d and e, agar films in cotton blue). Magnification: a, b, and c and d inset, 1500; d and e, 500.*

FIG. 4. *Conidia and chlamydospores of B. dermatiditis: a, conidia; b, stained mycelium, showing conidia and two chlamydospores. Magnification: 500.*

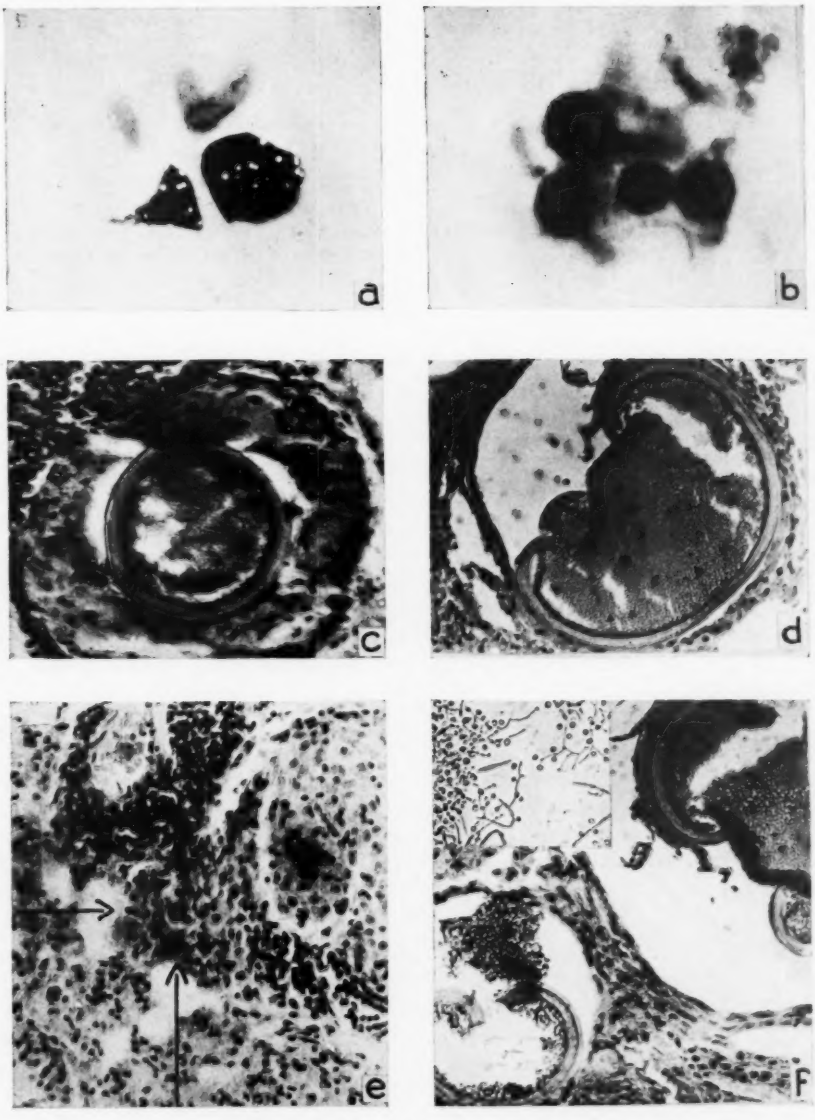

FIG. 5. *H. parvum in the mouse lung: a, infected lung embedded in paraffin, viewed with reflected light; b, similar lung from which paraffin has been dissolved, viewed in transmitted light; c, chlamydospore in the centre of granulomatous tissue; d, chlamydospore in lung that shows little reaction to the fungus; e, same lung as c showing very small fungous cell (arrows point to it); f, same as d with an inset showing, under the same magnification, the sporangia of H. parvum in culture. Magnification: a, 4; b, 50; c, d, e, and f, 150.*

TABLE I

RODENTS COLLECTED, AND THE NUMBER WITH *Haplosporangium* INFECTION OF THE LUNGS

Species	Number collected	Number infected
Citellus richardsonii (ground squirrel)	75	0
Clethrionomys gapperi athabascae (red-backed mouse)	1	0
Glaucomys sabrinus sabrinus (flying squirrel)	1	0
Mustela longicauda longicauda (weasel)	1	0
Microtus pennsylvanicus drummondi (meadow mouse)	33	0
Peromyscus maniculatus borealis (white-footed deer mouse)	117	13
Reithrodontomys humulis (harvest mouse)	25	0
Sciurus hudsonicus baileyi (red squirrel)	12	1
Total (8)	275	14

organisms. The fungi were therefore in all probability non-pathogenic Geotricha. During this survey, no *Coccidioides immitis* was discovered in any rodents, nor to our knowledge have any human cases of coccidioidal granuloma been described in Alberta. We conclude that the fungus is absent from this province.

Of the 275 animals examined, the lungs of 14 (13 white-footed deer mice and one red squirrel (Table I)) possessed pearl-like bodies. The bodies are illustrated in Fig. 5, *a* and *b*. Fig. 1 shows the localities from which the infected animals were collected. Most of them came from near Lethbridge but the infection is widely distributed over the province.

When planted upon agar, lung fragments of eight of these 14 animals produced growths of *Haplosporangium parvum* Emmons and Ashburn (9). The lungs of every one of these eight animals, when examined microscopically, were found to contain very large fungous cells. The range of *H. parvum* has therefore been extended into Western Canada, and two new hosts have been reported—*Peromyscus maniculatus borealis* and *Sciuris hudsonicus baileyi*.

Cultures of Canadian strains of *Haplosporangium parvum* are shown in Fig. 2 and agree with the descriptions of Emmons and Ashburn. The young mycelium of *Haplosporangium parvum* is moist and yeast-like. Later white tufts of aerial hyphae, which in profile are shown in *c*, form a chalky covering over the culture (*a* and *b*). Older cultures vary. They may become zoned concentrically (*h* and *i*). They may become convolute (*g*, *k*, and *l*). They vary in colour. The predominating colours are: in young cultures, white, pale olive gray, and pale olive buff; and in old cultures, buckthorn brown and cartridge buff (11). Many cultures produce sectors or tufts of chalky white mycelium (*e*, *j*).

The strain kindly sent by Dr. Emmons, isolated from an Arizona rodent, was much more fluffy than any of the Canadian strains.

Microscopically the Canadian strains agreed with the original description.

Haplosporangium parvum

The Sporangia (Conidia); Their Nature and Mode of Dispersal

Emmons and Ashburn (9) described the reproductive structure of *Haplosporangium parvum* as a conidium but suggested that it might be defined as a one-spored sporangium, sporangiole, or stylospore. They observed a hyaline body within it.

The following observations are presented as evidence for interpreting the conidia as sporangia.

1. When the mycelium of *Haplosporangium parvum* is examined after it has been stained in cotton blue and mounted in glycerine–eosin in a permanent Diehl preparation, the outer wall of the structure under question appears unstained, double-contoured, and spheroidal, while the inner body is blue-stained, single-contoured, and spherical (Fig. 3, *a*).

2. When 'conidia' are transferred to a hanging-drop of Sabouraud's medium, in about 20 hr. their outer walls loosen and the enclosed refractile spherical bodies become very distinct. A number of the refractile bodies germinate

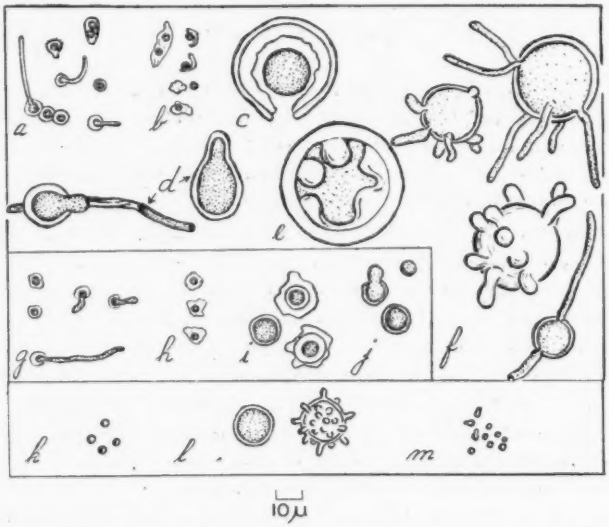

FIG. 6. *The spores of some pulmonary fungi: a to f, H. parvum: g to j, B. dermatiditis; k to m, H. capsulatum (a and g, germinating sporangia; b and h, sporangia in water with wall disintegrating; c, d, e, f, i, and l, chlamydospores; j, chlamydospores germinating).*

and the germ tubes break through the outer wall (Fig. 3, *d*, inset, and Fig. 6, *a*). When they are left in a water film instead of in agar for 24 hr. the outer wall not only loosens but dissolves (Fig. 6, *b*), freeing the inner refractile bodies.

It is concluded that the 'conidia' of *Haplosporangium parvum* are indeed sporangia. Usually they contain a single spore discernible in water or

glycerine–eosin mounts, but larger clavate sporangia may contain two or three spores (Fig. 3, *b* and Fig. 6, *a* (upper right)).

The following experiments with *H. parvum* were carried out.

1. Moist sterile soil was inoculated with the fungus. Within two weeks at room temperature it had grown and sporulated abundantly.

2. An agar culture was placed upside down over sterile agar. It was tapped repeatedly and allowed to remain inverted over the agar for some hours. No sporangia were discovered on the agar beneath. The sporangia are therefore not air-borne.

3. A culture was lightly touched with a sterile glass rod, and the rod then allowed to touch sterile agar. It left a heavy sporangial deposit. The sporangia must therefore be adhesive and are transferred by contact. Fig. 3, *d* shows a sporangium deposit that had been transferred by a glass rod three days previously. Some of the spores within the sporangia are germinating.

The experiments described above suggest that in nature *H. parvum* probably maintains itself in soil, and that burrowing rodents come into contact with the adhesive sporangia. When the sporangia reach their nostrils the sporangial walls may dissolve in the moist environment, and the sporangiospores become inhaled.

The Transformation of Incubated Sporangia into Chlamydospores

When cultures of *Haplosporangium parvum* that were growing at room temperature were kept for two weeks in an incubator set at 37° C., the filamentous growth began to be transformed into masses of large thick-walled spherical cells. Certain strains after two months' incubation became almost entirely converted into the unicellular condition. Because of the large size of these cells, the thickness of their walls, and the difficulty in germinating them, they will be referred to as chlamydospores. Chlamydospores may reach as much as 70μ in diameter. They are shown in Fig. 3, *c*, and Fig. 6, *c*, *e*, and *f*.

It can be seen by reference to Table II that at 37° C. isolates of *H. parvum* react differently to heat. Some Alberta strains (e.g. 10) were almost entirely converted to large chlamydospores. The Alberta strain $F^{2}9$ produced only a few small chlamydospores, while an Arizona strain was entirely unaffected by incubation.

The walls of most of the chlamydospores were 4 to 10μ thick, but Strain 17 possessed walls measuring up to 18μ almost obliterating the central cavity of the spore.

When agar is dabbed with the aerial mycelium of a culture of *H. parvum* and the sporangium print so obtained is incubated, it can be seen that it is the sporangia that enlarge to form chlamydospores, and indeed the point of attachment to the sporangiophore can still often be made out when the spores are in side view (Fig. 6, *c*). When the whole mycelium is incubated, certain cells in the hyphae swell and contribute to chlamydospore formation. Such chlamydospores often give a false appearance of budding (Fig. 6, *d*).

TABLE II

SIX STRAINS OF *Haplosporangium*

Source	Chlamydospores in incubated cultures	Chlamydospores in wild rodent lungs	Reaction in lung from which isolate was derived
Culture sent by Dr. Emmons from Arizona rodent	None; all the sporangia remain unchanged	Very small, measuring no more than 14μ (from published account)	No granulomatous reaction (from published account)
Mouse F29	Heat had little effect, but a few of the sporangia enlarged to 7μ in diameter	110μ	Slight reaction
Mouse 19	About a quarter of the sporangia grew to 10μ	Very large, averaging 240 μ; some reached 270μ	Very slight reaction
Mouse 184	Not incubated	150μ	Practically none
Mouse 190	About half of the sporangia grew to 25μ	150μ	Markedly granulomatous
Mouse 10	More than half of the sporangia grew to 40μ	150μ	Slight reaction (lungs from animals 10 and 17 accidentally placed in same bottle in the field)

Chlamydospores of *H. parvum* do not bud. They do not proliferate in any way. They merely enlarge. Neither has any endospore formation ever been observed in them.

Chlamydospores after being incubated at 37° C. for about three months were transferred to room temperature for a few weeks and then planted in hanging drops of soil agar and in hanging drops of Sabouraud's medium. After one and one-half days in both media about 75% of them began to germinate. The larger spores sent out 16 or more germ tubes, which penetrated the thick wall by a narrow channel and then enlarged and grew out radially (Fig. 3, *e* and Fig. 6, *f*).

These laboratory experiments suggest that when the sporangia of Canadian strains of *H. parvum* are inhaled by wild rodents they enlarge to chlamydospores in the warmth of the lung. Then, after the death of the animal the chlamydospores germinate in the soil and thus initiate the saprophytic phase of the fungus. It was to be expected that the Arizona strain could not be converted to chlamydospores at 37° C., because if it were so sensitive to heat it would not be able to maintain itself in the Arizona desert. It is of interest that the lungs of infected Arizona rodents contained only small fungous spores, while, as will later be shown, those of Alberta rodents contained very large ones.

Chlamydospores in Canadian Rodent Lungs

The lungs of 14 wild Alberta rodents, eight of which yielded *Haplosporangium parvum* in culture, were studded with large, spherical, pearl-like bodies easily visible to the naked eye (Fig. 5, *a* and *b*). These bodies, which were particularly evident after the organs had been embedded in paraffin, were fungous cells. They measured 110 to 270 μ in diameter.

In comparison, the lungs of infected Arizona rodents contained fungous cells averaging only 10 μ in diameter (9), although they were somewhat larger in experimentally infected animals (1).

The enormous size of the pathogens in the Canadian rodents is all the more remarkable when we consider that they have evidently developed from the inhaled sporangia of *Haplosporangium parvum*, which measure less than 4μ. It has already been demonstrated that the sporangia under incubation may grow to 10 times their original diameter, but in the lung they must have increased about 50 times. Fig. 5, *f* shows the fungous cells in the lungs, and, in the inset, the sporangia from which they must have originated, both under the same magnification.

The spores in the lung are taken to be the same type as those obtained by artifically incubated sporangia, and they too will be referred to as chlamydospores.

The wall of the lung chlamydospore may be as much as 8μ thick and usually appears to be in two layers, an outer thinner layer, perhaps the original sporangial wall, and an inner thicker layer, perhaps the original spore wall (Fig. 5, *c* and *f*). The cytoplasm towards the periphery of the chlamydospore is densely staining and reticular. Haematoxylin stain shows that at least some of the chlamydospores are multinucleate. The writer is unable to explain the evenly spaced clusters of granules frequently seen (Fig. 5, *d*). No endospores of *Haplosporangium parvum* have ever been observed in the lung.

Infected lungs possessed the normal alveolar pattern. The parasitic cells scattered through the organ were near the endings of the terminal bronchioles but were never within them. They were found in alveoli. Usually they were each surrounded by a narrow rim of compressed fibrous tissue (Fig. 5, *d*) giving the impression that the inhaled sporangium had first become lodged in an individual alveolus and there expanded, so that it compressed the adjacent alveoli.

In only one of 14 infected lungs was definitely granulomatous reaction observed (Fig. 5, *c*). Surrounding each fungous cell in this lung there was a band composed of masses of epithelioid cells, which was in turn surrounded by a narrow zone of lymphocytes. There were present a few foreign-body giant cells and scattered eosinophiles. The central chlamydospore was thick-walled and averaged 150 μ in diameter. In this lung a single small fungous cell was discovered of about the same size, 10 μ, as those in the Arizona rodents (Fig. 5, *e*).

Fig. 5, *d* shows lung tissue typical of the remaining 13 infected rodents. There was very little inflammation. The chlamydospores, about 200μ in diameter, were surrounded by a narrow rim of compressed fibrous tissue.

There seems to be no correlation between the size of the chlamydospores in an individual mouse lung and the size of the chlamydospores in the cultures derived from that lung. Thus Table II shows that the chlamydospores in the lungs of Animal 19 reached 270μ but those in incubated cultures from the same animal were not particularly large (about 10μ). Yet it must be admitted that, as seen from the table, the average diameter of the fungous cells in the lungs of Arizona rodents has been recorded as only 10μ and that cultures from such an Arizona rodent incubated in this laboratory did not change at all.

The name *Haplosporangium parvum* was given because the species is more delicate than the other members of the genus, but it is perhaps unfortunate for a fungus that is capable of enlargement to such gigantic size. Indeed, a more fitting description of it would be *multum in parvo!*

Relationship with Some Human Pathogens

Blastomyces dermatiditis Gilchrist and Stokes

Blastomyces dermatiditis is the organism responsible for North American blastomycosis (Gilchrist's disease or Chicago disease) in man. From the descriptions of it in the literature (4) and from studies of cultures in this laboratory, it can be seen to resemble the animal pathogen *Haplosporangium parvum* in the following respects:

1. The type of growth in culture varies with the temperature. At room temperature the fungus is composed of multicellular filaments; at 37° C. (either in the incubator or in the host), of unicellular spores (Fig. 7).

2. Macroscopically, the mycelium of *B. dermatiditis* resembles that of *H. parvum*. It grows out of the agar into the air in slender spines. Cultures may show a circular ring of clear agar devoid of mycelium. They are white at first, later becoming buff or brown.

3. Microscopically the mycelium is almost indistinguishable from *H. parvum*. It is septate. It produces hyphal fusions. The conidia (in the writer's opinion, sporangia (Fig. 4, *a*)) are borne in the same manner upon the conidiophores as are the sporangia of *H. parvum*.

4. The conidia are spherical or pyriform, similar to and only slightly larger than the sporangia of *H. parvum*. When old they also enlarge to form thick-walled chlamydospores (Fig. 4, *b* and Fig. 6, *i*).

The two fungi differ in the following respects.

1. At 37° C. *B. dermatiditis* reproduces by budding (Fig. 6, *j*); *H. parvum* does not bud or proliferate in any way.

2. *B. dermatiditis* produces chlamydospores at room temperature; *H. parvum* only at 37° C.

The following experiments similar to those already described for *H. parvum* were carried out in the laboratory with an American strain of *B. dermatiditis.*

1. Petri dishes of sterile soil were inoculated. Within two months a fluffy growth had covered most of the surface. The mycelium produced quantities of conidia.

2. The aerial mycelium of a culture was dabbed with a glass rod and the rod touched against agar in a plate. Microscopic examination of the agar showed a dense deposit of conidia.

3. Conidia were transferred to a film of water. Seven days later the outer walls of the conidia had loosened and become only faintly visible. Within could be seen a highly refractile spherical body (Fig. 6, *h*).

4. Conidia were transferred to Sabouraud's medium. Twenty hours later the outer wall of the spores had loosened and the inner bodies had germinated. (Fig. 6, *g*).

In all the behaviour above described—the growth on soil, the transfer of the conidia by contact, the separation of the conidial wall in water into an outer part and an inner body capable of germination—*B. dermatiditis* is identical with *H. parvum.*

Conant's figures of *B. brasiliensis* Conant and Howell (4) show a similar close relationship to *H. parvum.*

Histoplasma capsulatum Darling

H. capsulatum, the organism responsible for histoplasmosis in man, resembles the fungi previously described. Its habit of growth varies with the temperature (Fig. 7). The filamentous mycelium at room temperature produces similar conidia, which also enlarge into thick-walled chlamydospores (3) (Fig. 6, *l*).

Cultures of *H. capsulatum* obtained from the United States were found to grow and sporulate upon soil. A conidial print could be made on an agar film by placing the culture in contact with the agar. *H. capsulatum* differs from *Haplosporangium parvum* in that at incubator temperatures, and also within the host, the single cells reproduce by budding (Fig. 6, *m*), and it differs also in the distinctive spine-like ornamentations of the chlamydospore wall in culture.

Coccidioides immitis Rixford and Gilchrist

C. immitis is another member of the lower fungi that is filamentous in culture and unicellular in the host tissue (Fig. 7). Like the pulmonary fungi described above it is capable of growing on soil and has been isolated from soil in California. It is of particular interest in connection with *Haplosporangium*, because it also has been found in rodent lungs.

Emmons and Ashburn (9) found that when infected rodent lungs were examined microscopically, both *Coccidioides immitis* and *Haplosporangium parvum* appeared as variably-sized single spherical cells, sometimes surrounded

by granulomatous tissue. From 16 animals *C. immitis* only was isolated, from 92 animals *H. parvum* only, and from nine animals both *C. immitis* and *H. parvum*. Disregarding the nine animals with mixed infection, *C. immitis* was isolated from the granulomatous rodent lungs that contained the larger

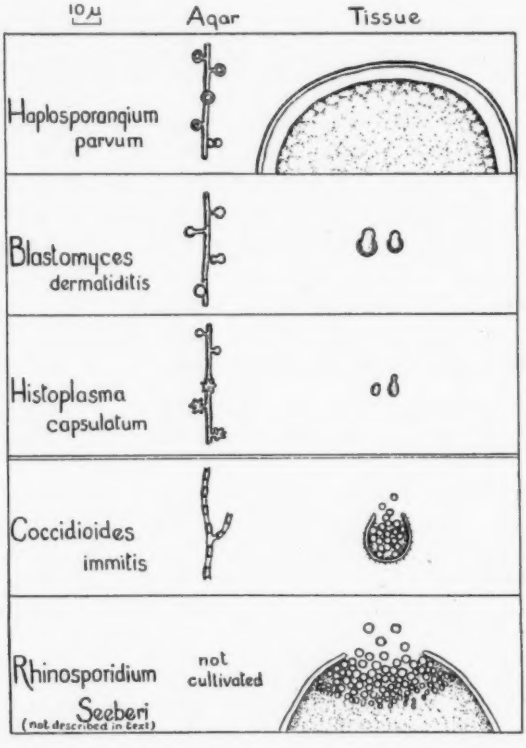

FIG. 7. *The saprophytic and parasitic appearance of Haplosporangium and four human pathogens.*

and more variable-sized fungous cells (up to 30 μ in diameter). *Haplosporangium parvum* was isolated usually from the non-granulomatous rodent lungs that contained the smaller and more constant-sized fungous cells (10 to 14μ in diameter) with denser-staining contents.

Emmons and Ashburn consider *H. parvum* and *C. immitis* very closely related and think it possible that *H. parvum* reproduces in the lung by endospores just as *C. immitis* is known to do. Some of the evidence for this is based on the immunological relationship of the two fungi, which was demonstrated by skin tests on young people.

The infected rodents were collected from a country in which at the time of the survey no cases of human coccidioidal granuloma had been recorded

but in which a large proportion of the population were sensitive to extracts of *Coccidioides immitis*. In San Carlos 27 Indian school children, who reacted in one arm to an antigen prepared from *C. immitis*, reacted similarly to an extract of *Haplosporangium parvum* injected into the other arm. This suggested to Emmons and Ashburn the possibility that the two fungi are closely related and that one might, in fact, be a mutant of the other. The present writer is unable to agree with either of these suggestions.

Since *C. immitis* has never been found in Alberta, and since *H. parvum* only has been isolated from infected rodents, the pathogen in the lung of the Canadian animals can be confidently identified as *H. parvum*. Canadian rodent lungs never contain endospores, nor have we found endospores in the *H. parvum* chlamydospores obtained by incubation.

Coccidioides immitis differs from the fungi described above in the following respects:

1. *C. immitis* grows on artificial medium into a mycelium that readily fragments to numerous infectious air-borne spores (oidia), readily blown about by the wind (Fig. 7). *Haplosporangium parvum*, *Blastomyces dermatiditis*, *B. brasiliensis*, and *Histoplasma capsulatum* reproduce themselves saprophytically by conidia (probably sporangia containing single endospores) that are not air-borne but distributed by contact. There is thus a fundamental difference in the mode of dispersal of the saprophytic phases of the two fungi.

2. *C. immitis* in the host exists as a single cell, or sporangium, which when mature discharges numerous endospores (sporangiospores) (2) (Fig. 7). The parasitic cells of *Haplosporangium parvum*, *B. dermatiditis*, *B. brasiliensis*, and *Histoplasma capsulatum* have never been observed to produce endospores.

C. W. Dodge (5) and others place *Coccidioides immitis* among the higher fungi (Ascomycetes). The parasitic cells they consider asci, and the spores within them, ascospores. With this opinion the writer is not in agreement. It is characteristic of an ascus that it forms its ascospores by 'free cell division' i.e. astral rays delimit the spores from the remainder of the cytoplasm (10). Such spore formation has not been demonstrated in *C. immitis*. Emmons (8), Baker, Mrak, and Smith (2), among others, are convinced that the endospores are cut out by cleavage planes in such a way that all the cell cytoplasm is included in the spores. Such spore formation is characteristic of the lower fungi (Phycomycetes).

We then have grounds for considering that *Coccidioides* resembles *Haplosporangium* in being a Phycomycete. Yet although both fungi produce lung granulomata, the writer is of the opinion that *Coccidioides* is not as closely related to *Haplosporangium* as is *Blastomyces* or *Histoplasma*.

It is quite possible that all the fungi under discussion constitute a natural group.

Conclusions

Haplosporangium parvum is certainly a Phycomycete. In the writer's opinion it closely resembles *Chaetocladium*. The human pathogens mentioned above, although not showing the morphological features of the Lower Fungi so clearly, resemble *H. parvum*. It is quite possible that all the fungi under discussion constitute a natural group.

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References

1. Ashburn, L. L. and Emmons, C. W. Arch. Path. 39 : 3-8. 1945.
2. Baker, E. E., Mrak, E. M., and Smith, C. E. Farlowia, 1 (2) : 199-244. 1943.
3. Conant, N. F. J. Bact. 41 : 563-578. 1941.
4. Conant, N. F. and Howell, A. J. Investigative Dermatol. 5 : 353-370. 1942.
5. Dodge, C. W. Medical mycology; fungous diseases of men and other mammals. C. V. Mosby Company, Medical Publishers, St. Louis. 1935.
6. Dowding, E. S. Mycologia. In press.
7. Emmons, C. W. Public Health Rept. 57 : 109-111. 1942.
8. Emmons, C. W. Mycologia, 34 : 452-463. 1942.
9. Emmons, C. W. and Ashburn, L. L. Public Health Rept. 57 : 1715-1727. 1942.
10. Fitzpatrick, H. M. The lower fungi. Phycomycetes. McGraw-Hill Book Co., Inc., New York. 1930.
11. Ridgway, R. Color standards and color nomenclature. Published by the author. Washington, D.C. 1912.

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